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The Damping Off of Coniferous Seedlings.

C. M. GIFFORD

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*In cooperation with Bu. An. Ind., U. S. Dept. Agr.

BULLETIN 157. THE DAMPING OFF OF CONIFEROUS SEEDLINGS

C. M. GIFFORD.[1]

SUMMARY

I. Several species of coniferous seedlings commonly grown in nurseries, viz.: white pine (Pinus strobus), Scotch pine (P. sylvestris), yellow pine (P. ponderosa), Norway spruce (Picea excelsa) and European larch (Larix decidua), are subject under unfavorable conditions to severe attacks of "damping off."

II. The damage may range from almost nothing to 90 percent of the stand, it being increased by warm weather following germination, by too much shading of beds, and by an alkaline reaction of the soil.

III. This damping off is caused by a fungus of the genus Fusarium.

IV. This fungus appears to be native to this country and very widely distributed.

V. The fungus has been repeatedly isolated from the roots of two-year-old seedlings of white pine, which showed in the beds a progressive dying, in some cases the bed being practically ruined.

VI. The fungus grows readily on all the varieties of cultural media used. It fruits abundantly, producing three kinds of spores.

VII. The spores first produced are called microconidia.

[1]The work upon which this bulletin is based was done by the late C. M. Gifford. His senior thesis, a prerequisite for graduation from the agricultural department of the university, dealt with this malady; and during the past summer he continued his studies thereon. Before his departure for a position at the West Virginia Station, he collected his data and wrote up the subject essentially in the way it is now presented. After Mr. Gifford's death, by drowning in mid-December, the task of preparing his manuscript for publication fell upon the writer of this note. The changes made by the editor are only minor ones and involve more the manner of presentation than they do the subject matter itself.

B. F. LUTMAN.

They are of an elongated oval shape, often slightly curved, and usually become two celled. The macroconidia are the characteristic Fusarium spores, from two to three times longer than broad, more or less pointed at the ends and curved and from two to five times septate. The chlamydo-spores are spherical in shape, produced rather sparingly, and usually under adverse conditions.

VIII. The sterilization of the soil by the use of live steam did not give results warranting its general adoption. It lessens the amount of moisture in the upper layers of soil, probably by diminishing the power of the soil to draw up moisture from below by capillarity.

IX. The best preventive measure seems to be the dis-infection of the soil by the use of a one-half to one percent solution of formalin, used at the rate of the three-fourths of a gallon per square foot. Afer treatment the bed should be allowed to air for at least a week before sowing the seed.

INTRODUCTION

With the present widespread interest in forestry and in methods of forestry reproduction has come so large a demand for seedlings for forest planting that the commercial nurseries have been unable to meet it. Furthermore these nurseries have quoted relatively high prices. Hence the establishment of state nurseries in several states which furnish seedlings for forest planting to landowners at, as nearly as may be, the cost of production.

The first seed beds in the Vermont State Nursery were sown in 1907. One of the problems with which the new nursery had to deal, almost from the first, was the disease known as "damping off" of the young white pine seedlings. When the weather conditions were favorable to its spread, it often caused serious damage, hence methods for combating it were sought. During the summer of 1907, Mr. F. V. Rand of the class of 1908 isolated the fungus causing the malady, and determined the value of various chemicals for the disinfection of the soil. This was done under the general direction of, and in cooperation with, Dr. Perley Spaulding of the Bureau of Plant Industry. During the summer of 1908 further work on soil disinfection was carried on by the station. In the spring of 1909, a study of the disease and of the fungus causing it was suggested by the station botanist (Prof. L. R. Jones) to the writer as a topic for senior thesis. The work was begun in the latter part of June, carried on during the summer as a part of the field work of the station, and prosecuted throughout the past college year with the council of the then station botanist and bacteriologist (Profs. L. R. Jones and H. A. Edson).

REVIEW OF LITERATURE

In 1892 Hartig (3)[1] described a disease very similar to the one present in the Vermont seed beds, which sometimes did much damage to pine and spruce in the Bavarian nurseries. He

[1]Numbers in parenthesis refer to bibliography, p. 171.

thought the fungus to be a Nectria, but in a later writing called it *Fusoma parasiticum.* As a remedial measure he recommended diminishing the surplus moisture by removing shade. If old beds were to be used, he advised sterilizing the soil by firing for two days.

In an earlier publication of this station, Jones (5) advanced the opinion that the Fusarium-like fungus was of widespread occurrence, and a native, rather than an introduced, species. He stated that the development of the disease seemed to depend upon conditions of temperature and moisture in the beds, the previous treatment of the soil, which may tend to increase or diminish its fungus content, and the use of fungicides or other substances upon the beds during the critical period. He found that disinfection of the soil with a dilute solution of formalin before sowing, lessened the seed germination from 25 to 50 percent but also lessened the damage from damping off from 90 percent in the untreated beds to 9 percent in the treated. He also recommended the practice of sprinkling the beds with hot sand.

Pollock (7) found that the damping off in soil secured from the government nurseries at Halsted, Nebraska, was caused by a Fusarium. After the seedlings damped off in his pots they were allowed to stand for seventy-five days from the time of the planting the seed, when the ascus fruit of a Nectria was observed scattered over the surface of the soil, though never on the decaying plants. It was found to be similar both to *Nectria thuyana,* found by Ellis on *Cupressus,* and also to *N. indigens* found on rocks in the Alps.

Hartley (4) finds in these same seed beds a Fusarium, a Rhizoctonia, and a Pythium, probably *P. de Baryanum,* all capable of causing damping off, but the last named the most destructive, with the Fusarium next. He states that the drying out of the surface moisture increased the damping off, giving as a possible explanation the theory that drouth weakens the plants and so predisposes them to attack.

OCCURRENCE OF THE DISEASE

As noted previously, Hartig (3) described what appears to be the same fungus attacking pine and spruce in the German nurseries. It was found in June, 1907, on seedlings of white pine from the New York state nursery at Saranac Inn. Hartley (4) reports a Fusarium as causing a damping off in the government nurseries at Halsted, Nebraska. In August, 1909, diseased seedlings of white pine (*Pinus strobus*), Scotch pine (*P. sylvestris*), yellow pine (*P. ponderosa*), Norway spruce (*Picea excelsa*), from the nurseries of the Northwestern Forestry Co., at East Haven, Connecticut, were examined by the writer, and the spores (macroconidia) of a Fusarium were found upon them all. A considerable damping off was noted in the state nursery at Burlington in June, 1907, in the beds of Norway spruce, yellow pine, Scotch pine, white pine, and European larch (*Larix decidua*).

The above evidence would seem to support the statement made by Jones (5) that the fungus is of widespread occurrence, and probably native to this country rather than introduced.

DESCRIPTION OF THE DISEASE

The observations made by the writer confirm those of earlier students of this malady as to method of infection by the damping off fungus. This seems to take place by the tip of a fungus thread boring its way into the tender stem of the seedlings, probably dissolving a portion of the epidermis by means of the secretion of an enzym. Having gained entrance to the soft internal tissues it grows vigorously, and in a short time so weakens the stem that the seedling falls over and, its water supply being cut off, soon dies. In some cases, especially when the seedlings are in a dense stand, and the weather conditions are very favorable, the fungus seems to attack the upper portion of the stem just at the base of the cotyledons or seed-leaves. Again, it has been observed that under certain conditions the fungus may attack and kill the seedling, and the attack be so general

that the seedling does not fall over, but remains standing, dry and shriveled. This was seen during the past summer in some beds of Scotch pine. At first it was thought that the death was due to other causes, perhaps a lack of moisture; but upon examining one of the seedlings under the microscope the long sickle shaped Fusarium spores, macroconidia, could be seen in great abundance. Therefore it was thought probable that the attack of the damping off fungus was largely responsible for the death of the seedlings.

FACTORS INFLUENCING THE AMOUNT OF DAMAGE

The amount of damage which may be caused by the fungus varies with the temperature and moisture conditions during the time following germination. Closely related thereto is the way in which the beds are handled. From the time of sowing until germination is completed, the beds must be covered to keep out the light and to conserve moisture. In the state nursery this is usually accomplished by means of a frame, with sides eight inches high, made of two 1x2 inch strips run parallel, the space between being covered with fine poultry netting to keep out the birds. This is covered by a frame of lath 4x12 feet, every other lath being removable, so as to give half shade after germination. At the time of sowing a strip of building paper is tacked around the sides, but is removed after germination unless the weather is extremely dry or the reverse. By means of this lath shade the amount of moisture in the surface layers of the soil can be very closely regulated.

Another factor, which seems to have an important bearing upon the amount of damage, is the reaction of the soil. At the time the state nursery was started, in the spring of 1907, a portion of the field was given a heavy dressing of lime-kiln ashes. Ever since that time the soil has been alkaline and in that portion of the nursery there has always been more loss from damping off than in the area not so treated. It has been found that with other diseases caused by fungi in the soil, as potato scab

and root-rot of tobacco, an alkaline reaction greatly increases the amount of injury.

While it is possible in the case of the damping off of the pines that the alkaline reaction has the direct effect of lessening the vigor of the host plant, it seems more probable that its effect is that of increasing the virulence of the parasite. From the excellent results reported by Spaulding (9) from the use of sulphuric acid in combating the disease it would seem that the effect of an acid reaction must have been the inhibition of the growth of the fungus rather than any stimulation of the host plant. During the past two seasons (1909 and 1910) there has been but little damage in the state nursery due to damping off. The rainfall has been slight during the critical period, and the beds have been fertilized with a compost of muck and stable manure, thus tending to keep the soil reaction acid. Furthermore, the shading has been so managed as to tend to correct any weather conditions likely to cause the spread of the disease.

THE FUNGUS ON ROOTS OF OLDER SEEDLINGS

On June 25, 1909, bed 14 of the two-year old white pine was being dug up, preparatory to reseeding. The bed showed a rather uneven growth, in some places the seedlings being stunted and even entirely dead, while in other parts they looked healthy and vigorous. It had been decided to transplant those which were healthy, and reseed the bed for disinfection trials. A growth of a grayish white fungus was noted upon the smaller rootlets. Some of these seedlings thus affected were taken to the laboratory and the fungus isolated in pure culture on July 3. Other seedlings were collected from the same bed ten days later and on July 15 a fungus isolated by laying the roots on a plate of synthetic agar. The mycelium was found growing two days later, and from the cortical portion of the root, radiating in all directions. This was similar to the fungus obtained previously.

The seedlings used included both large, healthy looking ones and the smaller stunted ones. The latter when collected

did not show any external growth of mycelium, due probably to the fact that they were so nearly dead.

Seedlings which showed the same appearance as those in bed 14, were taken on July 21 from beds 4, 9, and 10, of two-year old white pine. The fungus was obtained from each of these beds in the same way as in the previous case.

In bed 14 where the mycelium on the roots was first observed, some of the seedlings appeared especially large and vigorous. Samples were taken July 24 to see if they were infected by the fungus. The roots showed little or no external growth of mycelium. Seedlings were also collected from bed 20, containing one-year old Scotch pine. These seedlings appeared healthy, and showed no external growth of mycelium. Cultures prepared in the same way as previously gave the same fungus from both sources.

Nine beds of year-old white pine which were dug up on May 20 showed many seedlings betraying the same appearance as the stunted lots in the older beds. But in this case the dying seemed to be uniform and complete all over the bed. It seems probable that the root systems were so weakened by the attacks of the damping off fungus the preceding summer that the plants were unable to live through the winter. Such being the case, it was to be expected that there would be a large amount of damping off in the young Scotch pine with which the bed was replanted.

The addition of the compost, howeever, probably lessened the alkaline reaction of the soil, and the weather was rather cool and dry and unfavorable to the attacks of the disease; hence little of it appeared.

The injury to beds 4 and 14 had continually increased and consequently they were spaded up, preparatory to reseeding on July 26 and 31 respectively. Early in September most of the seedlings along the south side of bed 9 seemed to be seriously attacked. Some were entirely dead and others had made but little growth. Those in the north half appeared healthy and to have made a good growth.

All of these beds were located in the area which had received the lime-kiln ashes; (page 148). No such conditions were manifested in any of the beds at the south end of the nursery, where the soil was found to be acid. During the summer of 1910 one bed of year-old white pine at the south end showed along its south side a condition very similar to that above described. Seedlings were collected therefrom, their roots thoroughly washed, and the fungus isolated without difficulty. The bed was watched throughout the summer, but the disease did not seem to progress as it did at the north end, probably because of the acid reaction of the soil.

The genus Fusarium is not so virulently parasitic as are some fungi, and hence requires especially favorable conditions for the development of its facultative parasitism, in this case the condition being an alkaline reaction of the soil. During the past summer the Fusarium which is thought to cause the stem blight of the China aster, *Callistephus hortensis Cass.,* as noted by Duggar (1) has been reported in Vermont and seems to be very destructive wherever it gets a foothold. A Fusarium causing a similar disease of the garden pea (*Pisum sativum*) has also been found. Hence it is probable that under favorable conditions the Fusarium of damping off can attack the roots and stems of the older seedlings and sometimes even kill them.

Description of the Fungus

Methods of Obtaining Pure Cultures

As hitherto stated on page 145 the fungus was first obtained in pure culture at this station in June, 1907. It was isolated from seedlings of yellow pine (*Pinus ponderosa*) and carried through the summer, but unfortunately the cultures were lost. The description and drawings then made did not come into the hands of the writer until after the fungus had been again isolated, when they were found to agree closely with the later observations.

Seedlings of Scotch pine (*Pinus sylvestris*) which appeared to have damped off, were dipped in mercuric chlorid 1-1000,

rinsed in sterile water and with sterile forceps dropped on a freshly poured plate of synthetic dextrose agar (Plate I, figure 2). Another method used was to cut out a piece of the stem at the point of attack, and drop it on a poured plate, as above. Some whole seedlings also were dipped in mercuric chlorid, washed in sterile water, and then placed in a moist chamber.

Plate cultures were prepared from the seedlings placed in the moist chamber, and from the plate cultures first made. These cultures all showed the characteristic Fusarium spores after ten days. Plates were poured from these, and the process continued till pure cultures were obtained.

Isolations were obtained at various times during July, 1909, from four different lots of white and Scotch pine seedlings obtained from various parts of the nursery where the beds were infected. These cultures were all carried along together and were used in the later inoculation experiments.

MYCELIUM

The mycelium shows neither on the surface of the soil nor on the seedlings which are attacked. The growth in poured plates of synthetic agar or potato agar is white and of varying degrees of density above the surface of the agar. If the spores are sown thickly, vigorous spore production takes place by the second day with little growth above the surface. If, however, the spores are few in number producing only three or four colonies on a plate, a luxuriant white mycelium becomes evident, the radiating hyphae forming a sort of rosette growth extending peripherally comparatively slowly. The mycelium is from 3 to 5 microns in diameter, septate, abundantly so where fruiting is taking place, and is well filled with oil drops.

MICROCONIDIA

The mode of formation of the microconidia was studied from thickly seeded potato agar plates. By cutting out a piece about one centimeter square, and placing it on a cover glass

which was sealed over the depression in a hollow ground slide, the mount could be examined with the high power objective while fruiting went on undisturbed. The method by which the microconidia are formed in shown by figure 1. It seemed to correspond very closely to the method observed by Smith and Swingle (8) in *Fusarium oxysporum*. A lateral hypha grows out from one of the main filaments, the end swells slightly and the portion forming the microconidium is cut off by what Smith calls a "construction furrow." The microconidium lies along-side of the first, the process continuing till five or six are formed. After fruiting has gone on for sometime, the appearance under the low power objective resembles a bunch of bananas. The formation of microconidia begins in from 24 to 26 hours and lasts for about 48 hours. The size when grown in potato agar ranges from 3 to 5.3 microns in diameter, and from 9.4 to 17.8 microns in length. The most common size is about 3.5 by 12 microns. Germination takes place very readily. In potato agar (figure 2) germ tubes are put forth in from 5 to 8 hours. In distilled water several were found to be germinating at the end of an hour and half. A septum usually forms across the middle of the spore, just before or during germination.

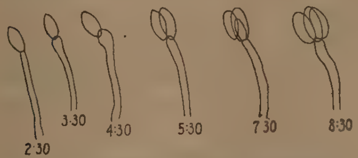

Fig. 1. Stages in the formation of microconidia of conifer Fusarium × 500.

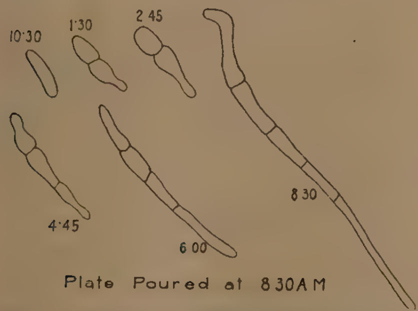

Fig. 2. Stages in the germination of a conifer Fusarium microconidium × 500.

MACROCONIDIA

It is by this type of spore that the Fusarium genus is recognized. They are produced about two days after the microconidia begin to be numerous, in older cultures being found almost to the exclusion of the former. They seem to be formed from short lateral branches above the surface of the medium, and are cut off in much the same way as the microconidia. They contain from one to five septa which appear after they have been set free from the parent branch. They are usually somewhat curved, especially toward the ends, which when grown in potato agar are not sharply pointed. In this medium the diameter was from 4.7 to 6.2 microns, and the length from 18.7 to 37.4 microns, the most common size being about 6 microns in diameter and 32 microns in length. When obtained from a diseased seedling, they were more slender and sharply pointed, and curved. Besides granular protoplasm, they usually contain oil drops. Their method of germination in hanging block cultures is shown in figure 3.

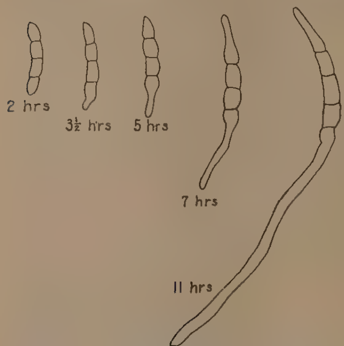

Fig. 3. Germination of a macroconidium × 250.

CHLAMYDOSPORES

The chlamydospores (figure 3) are spherical swellings of the mycelium, usually found at the ends of slender lateral hyphae, though they may be formed at any point in the hypha. They are thought to exist for the purpose of carrying the fungus through unfavorable conditions. They were first observed in some mycelium taken from a diseased seedling which had been placed in a moist chamber for two days. They were found very abun-


Fig. 4. Chlamydospores of conifer Fusarium × 500.

dantly in a growth of mycelium on the roots of some two-year old seedlings of white pine which had been placed in a moist chamber and after the mycelium had developed had been left until they dried. They were found in plate cultures where bacteria were numerous during the process of isolation of the fungus. On examining plate cultures up to a month old, chlamydospores were found fully developed in those 16 days old or older, the early stages being found in cultures 9 days old.

GROWTH IN DIFFERENT MEDIA

The fungus was grown in several different kinds of culture media, for the purpose of noting their effect upon its habit of growth, and with the possibility in mind of the discovery of the occurrence of the perfect stage in some medium not commonly used.

Synthetic dextrose agar. Growth rather dense, white, several millimeters high. In older cultures, surface of medium very much wrinkled and contorted.

Potato agar. Growth more rapid and luxuriant than above. At the point of inoculation in cultures two weeks old the macroconidia pile up in a cream-colored mass. Color white to cream-colored in older cultures.

Boiled rice. Mycelium at first white, but later lavender and salmon colored in spots. In darkness, mycelium on surface remains white.

Corn meal. Growth less rapid than on potato agar, but moderately dense. Mycelium white to grayish white in old cultures. In cultures two months old, small pustules about 2 mm. in diameter and 3 mm. in length were pushed out from the sides of the medium where it had dried away from the tube. When examined, they appeared to be made up mostly of macroconidia.

Boiled potato. Growth vigorous, completely covering cylinder at end of 11 days. At end of six weeks macroconidia would be piled up in grayish brown masses in different parts of the culture. No sclerotia were found as noted by Smith (8).

Lima bean agar. Growth even more vigorous than on potato agar. At end of 4 days a dense white growth completely covered the slant surface of 1.5 inches.

FIELD EXPERIMENTS WITH REFERENCE TO PREVENTIVE MEASURES

The work done during the summer of 1909 was with a view to testing out more fully the value of formalin in preventing damping off, and its effect upon germination of the white pine in the beds. It was also planned to determine the value of steam sterilization.

The trials were made in the north end of the state nursery. The land had been previously used as a vegetable and fruit garden and the first beds were sown in the spring of 1907. The soil is a sandy loam, containing considerable more humus than that at the south end of the nursery. At this time the land was given a dressing of lime-kiln ashes at the rate of fifty bushels per acre.

DISINFECTION WITH FORMALIN

Spaulding (9) in work of this sort obtained best results with dilute solutions of sulphuric acid, using one ounce of acid to one gallon of water. His results from the use of formalin at the rate of 4 ounces to 3 gallons of water were poor, in some cases the untreated plot being better. However, this may be explained by the fact that the seed was covered with untreated soil, while in the trials now under survey the soil used for covering was soaked over night in formalin solution and then dried before use.

The first trial was a simple test of formalin disinfection. On May 20 nine of the beds which had contained one-year-old white pine seedlings were dug up and eight of them sown to Scotch pine. It was thought at the time that the plants had been simply winter killed, but later development in neighboring beds pointed to the probability that death was in part due to the attacks of the damping off fungus on the roots.

Plate I. Figure 1. Pot grown seedlings of Scotch pine. Pot on left, sterilized; pot on right, unsterilized.

Figure 2. The mycelium (48 hours old) growing from a diseased seedling placed on a plate of synthetic agar.

PLATE II. Two-year-old seedlings of white pine, showing abundant mycelial development on the roots. Note the swollen appearance of many of the rootlets.

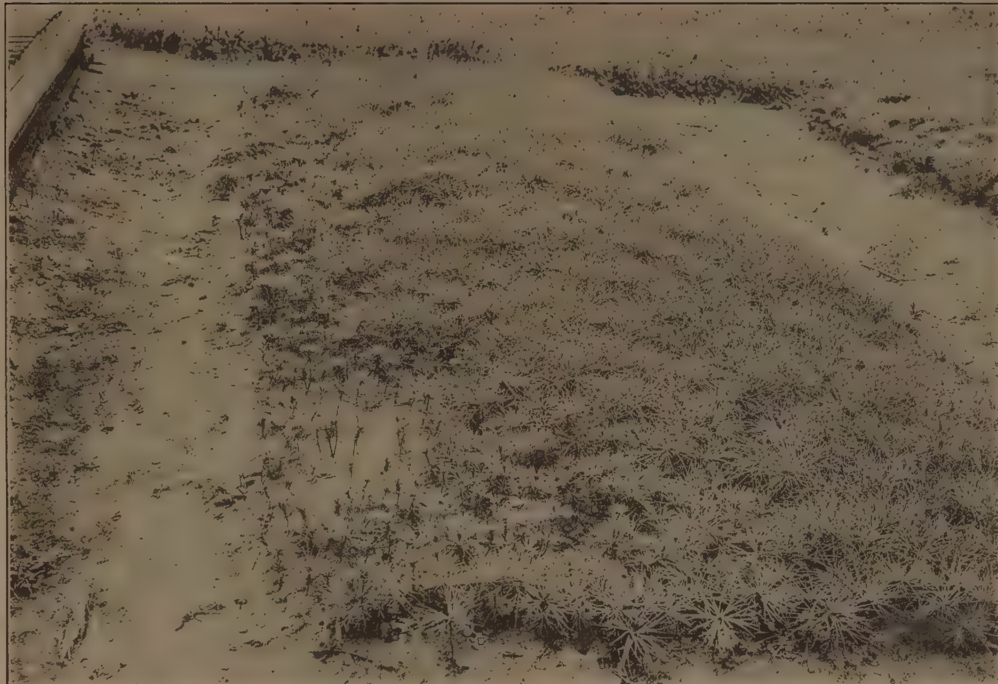

PLATE III. Bed of two-year-old white pine seedlings showing dying on one side of bed, due to damping off fungus.

PLATE IV. Figure 1. Untreated portion of bed of white pine seedlings, ten days after sowing. Figure 2. Treated portion (1% formalin) of similar bed.

On June 26 the ninth bed was spaded up and a wheelbarrow load of manure and muck compost thoroughly worked into the soil. The bed was then divided by board cross partitions into three equal parts and the middle one of these divided by a lengthwise partition into two equal parts, (figure 5). Formalin solu-

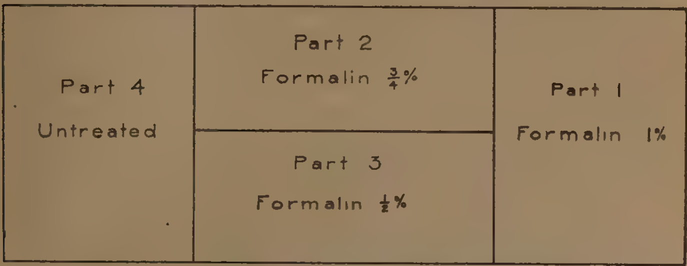

Fig. 5. Plan of bed used for formalin disinfection.

tion was then applied to two-thirds of the bed at the rate of 1½ gallons per square foot. The solution used on part 1 was made by adding one part of commercial formalin (40 percent formaldehyde gas in water) to 100 parts of water or a one percent solution. Part 2 received a three-fourths percent solution and part 3 a one-half percent solution. No water was added to the control. One-third of the formalin solution was added late in the afternoon of the 26th and the remainder on the morning of the 27th. The bed was enclosed by means of the building paper and the lath shades described on page 148, the latter being removed at the end of forty-eight hours.

On the afternoon of July 8, the bed was sown with 10 ounces of Scotch pine seed. The seed on parts 1-3 was covered with soil which had been disinfected by soaking over night in one-half percent formalin. The lath shades were again placed on the bed and left till germination was well under way.

On July 27 damping off had begun as shown by the following table:

Part.	Treatment.	No. of seedlings attacked.
1	1 percent formalin	13
2	¾ percent formalin	4
3	½ percent formalin	3
4	Control	50

On August 1, the disease had progressed considerably in the control, but showed little in the treated portions. Three days later about one-half of the seedlings in part 4 were attacked, while the other parts showed very little injury. From this time on, the disease continued to increase in the control until the 18th, when it was estimated that more than three-fourths were attacked, (see plate IV). At this time the disease seems to have nearly reached its height, for on the 24th its progress seems to stop, leaving the stand in the control perhaps one-tenth as good as that in the treated portions where the seedlings completely covered the ground.

The results of this trial show very clearly how serviceable formalin is when used on soil known to be infected with the damping off fungus.

LENGTH OF EXPOSURE AFTER USING FORMALIN

One of the objections to the formalin treatment is that it sometimes reduces the percentage of germination. In order to determine, if possible, the length of time it is necessary to leave the beds exposed after disinfection with formalin, in order to secure maximum germination from the white pine seeds, the following experiment was devised:

After being prepared for sowing in the usual way, a bed was divided lengthwise by a board partition into two equal parts. Each of these was then divided crosswise into four equal parts, making eight sections each 2x3 feet in size, (figure 6). Then one percent formalin solution was applied at the rate of one gallon per square foot to the entire half (A) of the bed, and the same amount of water to the other (B). The bed was covered with the complete lath shades and left for nearly 48 hours. At

the end of that time about 7 quarts of water were applied to parts 1a and 1b, and these were each sown with one and one-

4a	3a	2a	1a
4b	3b	2b	1b

Fig. 6. Plan of bed used in determining length of exposure after using formalin before sowing.

fourth ounces of Scotch pine seed. The seed on 1a was covered wih disinfected soil, and on 1b with untreated soil. This portion of the bed was left covered, and the loose lath were removed from the other three-fourths of the bed.

Two days later, parts 2a and 2b were sown in the same way and left covered. Parts 3a and 3b were sown on the 6th and parts 4a and 4b on the 10th. Thus it will be seen that 1a and 1b had no exposure after the bed was uncovered; 2a and 2b two days; 3a and 3b four days; and 4a and 4b eight days. Excepting for the use of formalin the two halves, a and b, received exactly the same treatment.

The seedlings in the "b" parts came up slightly ahead of those in the "a" parts, and a marked difference in size could be seen for a few days; but the latter soon caught up. There was considerable damping off in parts 1a and 2a, but there was far more in 1b and 2b, where considerably more than one-half the stand was killed. About one-third were attacked in 3b and one-fourth in 4b. Very little occurred in 3a and 4a. Three weeks later the total number of seedlings in a strip three inches wide was counted across each section except 1b and 2b where the number of damped off seedlings was so great that it could not be determined. The figures are given in the following table:

Part.	Number of seedlings.	Part.	No. of seedlings.
1a	214		
2a	194		
3a	177	3b	206
4a	163	4b	189

These figures show that the formalin reduced the percent of germination; but in no case does this reduction exceed 14 percent, while the loss from damping off in this case ranged from 25 percent to 75 percent and has been known to reach 90 percent. There seemed to be no great diversity in results which could be attributed to the different lengths of exposure before seeding, but in general, it would seem that a week would not be too long a period to elapse between treatment and seed sowing.

STEAM STERILIZATION

The sterilization of the soil by the use of live steam has been rather widely advocated for killing undesirable soil organisms. It has been used with special success in the treatment of tobacco beds for root-rot (*Thielavia basicola*). Gilbert (2) states that its advantage over the use of formalin consists in killing weed seeds, altering the physical texture of the soil making it more suitable for root development, and rendering considerable plant food dirctly available to the seedlings.

Lyon and Bizzell (6) found that steaming soil under pressure reduced nitrates to nitrites and to ammonia, and that the ammonification and nitrification processes were practically nonexistent during the three months following steaming. In their experiments steaming appeared so to loosen the soil, that when water was applied most of it percolated to the bottom of the pot, leaving the upper soil somewhat dry. At first wheat plants did not thrive as well in the steamed soil, but after a time they began to improve and made eventually a much more luxuriant growth than did those on the unsteamed soil.

The method of steam sterilization recommended by Gilbert is called the inverted-pan method. With some slight changes to meet the conditions encountered it was used in the trials now under survey.

Bed 17 was spaded up on July 5 and prepared for sowing in the usual way, except that the frame was not put in position. The bed was divided by crosswise partitions into three equal

parts (figure 7) the next day and one-half percent formalin solution was applied to part 2, at the rate of one-half gallon per

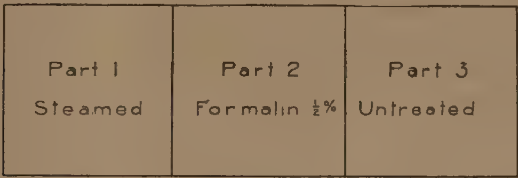

Fig. 7. Plan of bed used in steam sterilization of soil test.

square foot and the same amount of water to part 3. The bed was left uncovered. Two days later part 1 was steamed by use of a portable boiler. A galvanized iron pan, four and one-half feet square and eight inches deep was inserted over the bed, the edge being sunken into the soil to a depth of four inches. A thermometer was inserted horizontally under one corner. Raw potatoes were buried at depths of one, two and three inches before placing the pan in position.

When the pressure of steam in the boiler had reached 80 pounds, the valve leading to the pan was opened, and 27 minutes later the thermometer registered 201° F. The temperature remained at or near that point for half an hour, when it was found necessary to shut off the steam as the boiler was nearly empty. When the pan was removed six hours later, the potatoes were found to be thoroughly cooked.

The bed was then prepared and sown in the usual way using Scotch pine seed, which, in the treated parts, was covered with disinfected soil, and in the control with untreated soil.

Germination in the steamed part was a little slower than that in the other two parts, probably because of a lessened amount of moisture. The number of seedlings attacked on July 29 in the different parts was as follows:

Part.	Treatment.	Number attacked.
1	Steam	143
2	Formalin	105
3	Control	260

On this date was noted for the first time a difference in the amount of surface moisture in the different parts. Part 1 was dry over about five-sixths of its area, part 2 over nearly one-half, while part 3 appeared uniformly moist. This difference could be seen for several weeks, except for a short period following a rain.

From July 29 to August 3 the disease spread with great rapidity, and on the latter date the diseased seedlings were removed and either counted or estimated. The results are given below:

Part.	Treatment.	Number attacked.
1	Steam	1000
2	Formalin	579
3	Control	2000

The damping off continued to increase in parts 1 and 3, but there was little further damage in part 2. The loss in the steamed area was estimated two weeks later at 33 percent, in the formalined area at 10 percent, and in the untreated area about 50 percent of the total stand. The progress of the disease seemed to be stayed by the last week in the month.

SECOND TRIAL OF STEAM STERILIZATION

A bed was prepared on July 26 for sowing in the usual way. It was then divided by two crosswise partitions into three equal parts, and the middle one of these divided by a lengthwise partition (figure 8).

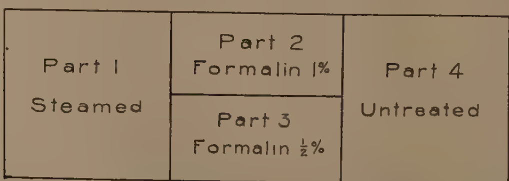

Fig. 8. Plan of bed used in comparing effects of formalin and steam sterilization on damping off.

Formalin solution was then applied to parts 2 and 3 at the rate of three-fourths of a gallon to the square foot. On part 2

a 1 percent solution was used, and on part 3-a ½ percent solution, an equal amount of water being applied to part 4.

Two days later the galvanized pan was placed over part 1 and steam passed in for an hour. The temperature, as registered by a thermometer placed under one corner was at, or near 212° F, for a period of forty-six minutes. The pan was removed after 48 hours and the bed sown in the usual way, with Scotch pine, the frame and tools having been washed with ½ percent formalin.

Germination was well started by August 25, but was progressing more rapidly in part 4 than in the other parts, especially part 1. This appeared to be due to a difference in the moisture content, as was observed in the previous experiment. This was especially noticeable after the extra shading had been removed, the surface of part 1 being uniformly dry, while that of part 4 was uniformly moist.

Damping off appeared in all parts, but was worst in part 4, part 1 being next. There was almost no loss in parts 2 and 3. Weather conditions at this time did not appear to be favorable to the development of the disease, for the loss in part 4 did not exceed 10 percent. A slight amount of injury was noted up to October 1, but more in part 4 than in part 1.

The results of these experiments would indicate:

1. That sterilization of the soil by the use of live steam is less effective in preventing the attacks of the damping off fungus than is disinfection of the soil by the use of a dilute solution of formalin.

2. That steaming lessens the amount of capillary moisture in the upper layers of soil.

The greater efficiency of the formalin treatment may be due to the fact that it leaves the soil less receptive to later infection than does steaming. In a steamed soil nearly all micro-organisms are killed and the first fungus which is introduced has an excellent opportunity for growth. Under ordinary nursery

conditions it is almost impossible to prevent the introduction of the spores of the damping off Fusarium after disinfection. Hence the method which not only kills or stops the growth of the fungus already in the soil, but also leaves the soil in a better condition to withstand its later introduction, is the method to be recommended. The slight value which steaming seems to have had may very likely be traceable to its effect in lessening the amount of surface moisture, fully as much as to its value in killing the fungus previously present in the soil.

THE EFFECT OF STEAM STERILIZATION UPON THE MOISTURE RELATIONS OF THE SOIL

The effect of steaming upon the moisture content of the surface soil caused so much speculation that a simple trial was made to determine the effect of steaming upon soil capillarity.

A quantity of air-dry soil taken from the nursery where the sterilization trials were held, was heated in the autoclave for 45 minutes at 100° C. Two heavy glass tubes about six feet long and one inch in diameter were then filled with the soil, tube No. 1 being left loose, and No. 2 being slightly compacted by shaking. In the same way No. 3 and 4 were filled with the same soil, unsteamed. Pieces of cheese cloth were tied over the lower ends of the tubes, which were set in a shallow dish, containing about an inch of water. The height to which water rose was noted for intervals for a period of two months.

From the outset the rise in the unsteamed soil was much more rapid, the water column averaging about twice as high as in the steamed soil. The platted curve (figure 9) shows the height and rapidity of rise in the two kinds of soil. At the end of 63 days the heights in centimeters in the four tubes were as follows:

No. 1	35.
No. 2	29.8
No. 3	75.
No. 4	79.5

At first sight the effect of steaming might be thought to be desirable, since it induces physical conditions unfavorable to the development of the fungus. A supply of moisture in the sur-

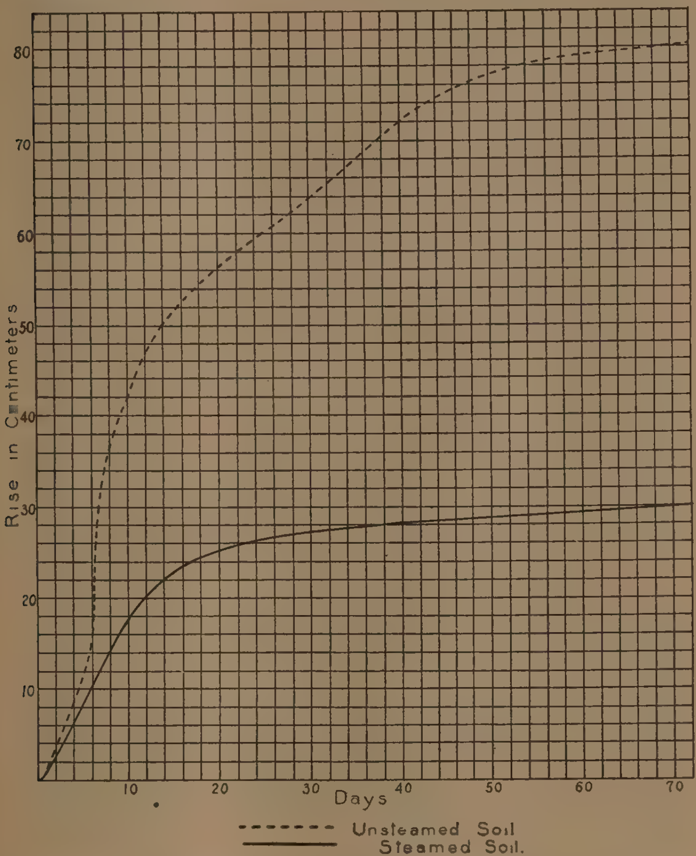

Fig. 9. Curve showing rise of water in steamed and unsteamed soil.

face layers of soil is essential, however, to good germination and to healthy growth. In fact, Hartley (4) states that the drying

out of the surface moisture *increased* the damping off. This, however, has not been the case under the conditions of the trials now under survey. The effect of steaming on the moisture relations of soil should be borne in mind by those using it in general greenhouse practise.

INOCULATION EXPERIMENTS

The inoculation experiments were undertaken for the purpose of determining if possible the method of attack of the fungus and the extent to which the reaction of the soil influences the virulence of the disease.

From a number of preliminary experiments which consisted in growing seedlings in pots of sterilized soil in the greenhouse, inoculating with pure culture of the damping off fungus, and recording the amount of loss from damping off, the field observations as to the relation between soil alkalinity and disease development were confirmed. Incidentally the results also indicated that the fungus is of very common occurrence in the greenhouse soil, for it was several times isolated from seedlings which had damped off in the uninoculated pots. These pots were thought to have become infected at the time of watering, or by insects crawling over them.

The soil used in the experiment was from the nursery, sandy in character and slightly acid to litmus. The plan of the experiment was as follows:

Pots no.	Treatment of soil.	Treatment of pots.
1—2	Reaction unchanged	Not inoculated
3—5	Reaction unchanged	Inoculated
6—7	Plus 4 grs. hardwood ashes	Not inoculated
8—10	Plus 4 grs. hardwood ashes	Inoculated
11—12	Plus 8 grs. hardwood ashes	Not inoculated
13—15	Plus 8 grs. hardwood ashes	Not inoculated
16—17	Plus ½ cc. sulphuric acid sp. gr. 1.82	Not inoculated
18—20	Plus ½ cc. sulphuric acid sp. gr. 1.82	Inoculated

After the pots were filled with the various mixtures of soil, a piece of wrapping paper was tied over the top of each

one, and they were sterilized in the autoclave for 1½ hours at 120° C. The seed was soaked for 10 minutes in a 1-1000 solution of mercuric chlorid, rinsed in sterile water, and dried on sterilized filter paper. The seed in each pot was covered with soil treated in the same manner as that in the pot. The pots were set on a freshly painted wood surface which was washed with mercuric chlorid. Each pot was set in a saucer which had been sterilized, and each two pots were covered by an open top bell-jar which had been wiped out with mercuric chlorid and the opening plugged with sterilized cotton. The jars were sealed to the shelf with a wax mixture to keep out insects. To provide a method of watering, a glass tube was introduced through the upper opening of the bell-jar into the saucer of the pot containing the seedlings. Water could be siphoned over into the saucer from the bottle in which it had been sterilized without serious danger of contamination.

The inoculations were made on April 15 by shaking up a small piece from a plate culture of the fungus in sterile water, the water containing the spores being poured over the soil of the pot to be inoculated. The following day, damping off appeared in three of the pots, and two days later on nearly all those inoculated. The bell-jars appeared to furnish conditions favorable to the rapid action of the fungus.

On April 23, diseased seedlings or growths of fungi from the different inoculated pots were examined, and the spores (macroconidia) of the damping off fungus were found in great abundance. The seedlings in the uninoculated pots remained healthy. The pots were not watered after April 30, and little attention was paid to them, as the two weeks was thought to be a sufficiently long time for the fungus to produce results. On May 31 the pots were examined and the seedlings were found to be dead in all the inoculated pots but those receiving the acid which were still healthy as were all but two of the uninoculated pots. The numerical results of this experiment are given in the following table:

Pot number.	Number germinated.	Number attacked within 5 days.	Average percent loss at the end of 5 days.	Average percent loss at the end of 15 days.	Pot number.	Number germinated.	Number attacked within 5 days.	Average percent loss at the end of 5 days.	Average percent loss at the end of 15 days.
1	30	24	26.6	80.0	13	19	19	68.4	100.0
2	26	22	42.3	84.6	14	18	17	72.2	94.4
5	10	8	30.0	80.0	17	14	8	28.5	57.1
8	21	17	47.2	80.9	18	33	21	21.2	63.6
9	13	13	46.1	100.0	19	32	26	6.2	81.2
10	34	33	38.2	97.0	20	25	17	4.0	68.0

SUMMARY OF ABOVE TABLE

Treatment of soil.	Average percent loss at the end of 5 days.	Average percent loss at the end of 15 days.
Reaction unchanged	32.9	81.5
Plus 4 grs. hardwood ashes	43.8	92.6
Plus 8 grs. hardwood ashes	70.3	97.2
Plus ½ cc. sulphuric acid	14.9	67.4

On account of the very favorable conditions created by the use of the bell-jars, the disease was very active, so that the figures at the end of 15 days do not show the differences that are shown in the experiments. But the results do indicate that an alkaline reaction of the soil favors the development of the disease and that, within limits, increasing the alkalinity increases the virulence of the attack. The relation of this fact to nursery practice would suggest the importance of the use of acid fertilizers and the danger in using those containing large amounts of alkali. It has not been determined whether this effect of an acid reaction is due to an increase in the vigor of the host or to a less favorable condition for the parasite, although the writer is inclined to accept the latter view.

EFFECT OF FERTILIZATION ON THE VIRULENCE OF THE DISEASE

In the light of the results previously secured, it was thought advisable during the summer of 1910 to test the effect of vari-

ous chemical and animal fertilizers upon the virulence of the disease under actual nursery conditions. It was hoped in this way to find a fertilizer which would be safe to use, or at least to find those which it would be best not to use.

On July 6 two beds were prepared for sowing. A pailful of infected soil was sprinkled over them, and very thoroughly raked in. Both beds were then divided lengthwise by board partitions, and to half of each bed was applied air-slaked lime at the rate of 2,000 pounds per acre. The lime was applied to the north half of bed 1 and to the south half of bed 2, and was carefully raked in. On July 9 the soil from various places on each side was tested with litmus and found to be alkaline where the lime had been applied, and neutral in the other half of the bed. Each bed was divided crosswise into six equal parts which were numbered consecutively from 1 to 12 (figure 10).

1	2	3	4	5	6
7	8	9	10	11	12

Fig. 10. Plan of bed used in testing effect of various fertilizers on damping off.

On July 18 fertilizers were applied to the different parts, according to the scheme given below. The small amount of the chemical used being previously in each case mixed with a quantity of the soil, so as to make possible an even distribution.

Part.	Fertilizer.	Rate per acre.
1	Control	
2	Sodium nitrate	200 pounds
3	Tankage	400 pounds
4	Potassium carbonate	400 pounds
5	Potassium chlorid	400 pounds
6	Acid phosphate	1000 pounds
7	Control	
8	Base goods	1000 pounds
9	Complete fertilizer (2, 5, 6 above)	1600 pounds
10	Hardwood ashes	2000 pounds
11	Lime kiln ashes	2000 pounds
12	Compost	1qt. per sq. ft.

The two beds were sown with Scotch pine on July 29. Germination was moderately good, and damping off was present throughout the two beds. The seedlings were counted August 16 after germination was completed, and again on September 12, when damping off had practically ceased. The figures are given in the following table:

Part.	Total germination.		Total stand Sept. 12.		Number attacked.		Percent attacked.	
	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed.
1	670	914	369	630	301	284	44.9	31.0
2	682	981	392	613	290	368	42.5	37.5
3	1239	1240	1109	1177	130	63	10.4	5.0
4	533	1065	311	767	222	298	41.6	27.7
5	688	1076	333	736	355	340	51.6	31.6
6	821	939	516	665	305	274	37.1	29.2
7	687	750	411	517	276	233	40.1	31.0
8	806	726	445	474	361	352	44.7	34.6
9	751	932	484	558	267	374	35.5	40.1
10	469	599	266	379	203	220	43.3	36.8
11	547	513	340	321	207	192	37.8	37.7
12	729	745	527	629	202	116	27.8	15.5

As will be readily seen, the above figures show with one exception a consistent increase in the amount of damping off where the soil was limed. This accords very well with the earlier findings. There appears to be no striking difference between the different fertilizers except in the case of part 3 which received tankage. This part gave excellent germination, and a very small amount of damping off. This material should receive further study as it may prove to be of real value in lessening the disease. The next best part appears to be the one receiving the compost. This material is now regularly used in the nursery seed beds and usually gives excellent results. It is made by composting together well-rotted horse manure and muck, half and half. Besides the plant food contained, it probably carries considerable amounts of organic acids, and its use would seem to approximate more closely the conditions under which the seedlings naturally grow, than the use of chemical fertilizers.

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